REMARK

The Commissioner is authorized to charge Lyon & Lyon Deposit Account No. 12-2475 for any fees necessitated by this filing.

If Applicants can help in any way to expedite this application, please contact the undersigned.

Respectfully submitted,
LYON & LYON LLP

Dated: 4/17/02

By: _____
Michael J. Wise
Reg. No. 34, 047

LYON & LYON LLP
633 W. Fifth Street, Suite 4700
Los Angeles, CA 90071
Ph: (213) 489-1600
Fax: (213) 955-0440

MARKED-UP COPY OF AMENDED CLAIMS

We claim:

1. (Amended) A method for determining if a test compound induces uracil misincorporation [UTP] into DNA, the method comprising:

a) providing aliquots of the following cells:

i) wildtype cells;

ii) cells overexpressing dUTPase [a duties];

iii) cells overexpressing a uracil-DNA [UT1] glycosylase; and

iv) cells expressing the uracil-DNA [UT1] glycosylase inhibitor protein Ugi or cells possessing a compromised uracil-DNA [UT1] glycosylase function;

b) exposing the cells to an agent that directly or indirectly inhibits thymidylate metabolism, in the presence or absence of the test compound;

c) measuring one or more features of the exposed cells, the features comprising:

i) cell growth or viability;

ii) cell cycle checkpoint arrest;

iii) presence of replication intermediates in the cells;

iv) amount of dUTP [DTP] present in the cells; and

v) presence or amount of uracil in DNA of the cells; and

d) interpreting the measured features, wherein a profile in the four cell types which is indicative that the test compound induces uracil mis-incorporation into DNA comprises one or more features in each of the cell types comprising:

i) in the wildtype cells, cytotoxicity, cell cycle arrest at G1/S or early S phase, presence of replication intermediates, elevated dUTP [DTP] pools or little or no detectable uracil in the DNA;

ii) in the dUTPase [duties] overexpressing cells, enhanced resistance to cytotoxicity, cell cycle arrest at mid S-phase, presence of replication intermediates, low dUTP [DTP] pools, or little to no detectable uracil in DNA

iii) in the uracil-DNA [UT1] glycosylase overexpressing cells, cytotoxicity or enhanced cytotoxicity, cell cycle arrest at G1/S or early S-phase, presence of replication intermediates, elevated dUTP [DTP] pools, or little to no detectable uracil in DNA; and

iv) in the nonfunctional uracil-DNA [UT1] glycosylase cells, enhanced resistance to cytotoxicity, cell cycle arrest at G2/M phase, reduced presence of replication intermediates, elevated dUTP [DTP] pools, or stable uracil incorporation into DNA.

5. (Amended) The method of claim 1, wherein the cells overexpress a dUTPase [duties] from an organism selected from the group consisting of humans, animals, plants, fungi, algae, protozoa, bacteria and viruses.

6. (Amended) The method of claim 1, wherein the cells overexpress a uracil-DNA [UT1] glycosylase from an organism selected from the group consisting of humans, animals, plants, fungi, algae, protozoa, bacteria and viruses.

7. (Amended) The method of claim 1, wherein the cells lacking a uracil-DNA [UT1] glycosylase function are produced by producing in the cells an inhibitor of uracil-DNA [UT1] glycosylase.

8. (Amended) The method of claim 1, wherein the inhibitor of uracil-DNA [UT1] glycosylase is obtained from a virus.

9. (Amended) The method of claim 1, adapted for determining if the test compound inhibits dUTPase [duties], the adaptation comprising, in step (d), observing in each of the four cell types one or more features comprising:

i) in the wildtype cells, cytotoxicity, cell cycle arrest at, G1/S or early S phase, presence of replication intermediates, elevated dUTP [DTP] pools, or little or no detectable uracil in the DNA;

ii) in the dUTPase [duties] overexpressing cells, continued growth resistance to cytotoxicity, cell cycle arrest not present or, if present, occurring at mid S-phase, presence or absence of replication intermediates, low dUTP [DTP] pools, or little to no detectable uracil in DNA;

iii) in the uracil-DNA [UT1] glycosylase overexpressing cells, cytotoxicity or enhanced cytotoxicity, cell cycle arrest at G1/S or early S-phase, presence of replication intermediates, elevated dUTP [DTP] pools, or little to no detectable uracil in DNA; and

iv) in the uracil-DNA [UT1] glycosylase inhibitor (Ugi) expressing cells, enhanced resistance to cytotoxicity, cell cycle arrest at G2/M phase, reduced presence of replication intermediates, elevated dUTP [DTP] pools, or stable uracil incorporation into DNA.

11. (Amended) A kit comprising:

a) aliquots of the following cells:

i) wildtype cells;

ii) cells overexpressing dUTPase [a duties];

iii) cells overexpressing a uracil-DNA [UT1] glycosylase; and

iv) cells lacking a uracil-DNA [UT1] glycosylase function; and

b) instructions for using the cells in an assay to determine if a test compound induces uracil misincorporation [UTP] into DNA.

12. (Amended) A method for determining the effectiveness in a patient of chemotherapy targeting conversion of dUMP to TMP, the method comprising:

a) obtaining from the patient a sample of cells which are the target of the chemotherapy;

b) measuring one or more features of the cells, the features comprising:

i) cell growth or viability;

ii) cell cycle checkpoint arrest;

iii) presence of replication intermediates in the cells;

vi) amount of dUTP [DTP] present in the cells; and

vii) presence or amount of uracil in DNA of the cells; and

c) observing if one or more of the measured features is the same as or differs from features comprising: cytotoxicity, cell cycle arrest at G1/S or early S-phase, presence of replication intermediates, elevated dUTP [DTP] pools or little or no detectable uracil in the DNA, wherein a lack of divergence from one or more of the features is indicative that the chemotherapy is effective, and a divergence from one or more of the features indicates a possibility that the chemotherapy is of reduced effectiveness or is ineffective.